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Phallus hadriani and *P. roseus* from Pakistan

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Abstract — Specimens of *Phallus hadriani* and *P. roseus* collected in Pakistan are described here. Photographs and molecular data are provided for both species.

Key words — *Basidiomycota, Phallales,* taxonomy.

Introduction

The genus *Phallus* Junius ex L. (*Phallaceae* Corda, *Phallales* E. Fisch.) is typified by *P. impudicus* L. Kreisel (1996) has recognized 31 different species within *Phallus* sensu lato (incorporating *Aporophallus*, *Dictyophora*, *Echinophallus*, *Endophallus*, *Itajahya*, and several other genera). Calonge (2005) proposed a provisional key for 25 species, after which several new species have been described (e.g., Calonge et al. 2005, 2008; Li et al. 2005; Moreno et al. 2009).

Phallus has a cosmopolitan distribution (Kreisel 1996); four species previously recorded from Pakistan include *P. celebicus* Henn., *P. impudicus*, *P. rubicundus* (Bosc) Fr. (Ahmad 1952), and *P. calongei* G. Moreno & Khalid (Moreno et al. 2009). Descriptions for two additional species based on recent collections from Pakistan are provided below.

Materials & methods

The collections preserved in LAH herbarium were examined and divided, with separate vouchers being deposited in AH herbarium. Specimens were mounted in Hoyer's medium and studied with a Nikon microscope. Spores were measured under the oil immersion objective.

DNA was extracted and amplified as described in Alvarado et al. (2012). The primers ITS1F and ITS4 (White et al. 1990, Gardes & Bruns 1993) were used to amplify the

Fig. 1. *Phallus hadriani:* Mature fruiting bodies with an egg. Bar = 0.5 cm

internal transcribed spacer region. Sequences were visually inspected for reading errors in MEGA4.0 (Tamura et al. 2007). Validated sequences were stored in GenBank under the accession numbers listed below. These sequences were aligned with the closest matches obtained with BLAST searches through the public databases. ITS sequences came mainly from Cheong (unpublished). Sequences were first aligned in MEGA 4.0 software using its ClustalW application and then corrected manually. The aligned locus was loaded in PAUP* 4.0b10 (Swofford 2001) and a maximum parsimony phylogenetic tree was reconstructed (2000 bootstrap replicates, TBR swapping algorithm, 50 sequence additions per replicate, MulTrees not in effect). It was also subjected to MrModeltest 2.3 (Nylander 2004) in PAUP* 4.0b10. The best model was then implemented in MrBayes 3.1 (Ronquist & Huelsenbeck 2003), where a Bayesian analysis was performed (5.8S-ITS2 data partitioned, 2 simultaneous runs, 6 chains, temperature set to 0.2, sampling every 100th generation) until convergence parameters were met after about 200,000 generations. Significance thresholds were above 70% for bootstrap (BP) and 99% for posterior probability (PP).

Taxonomy

Phallus hadriani Vent., Mém. Inst. nat. Sci. Arts 1: 517 (1798) Fig. 1

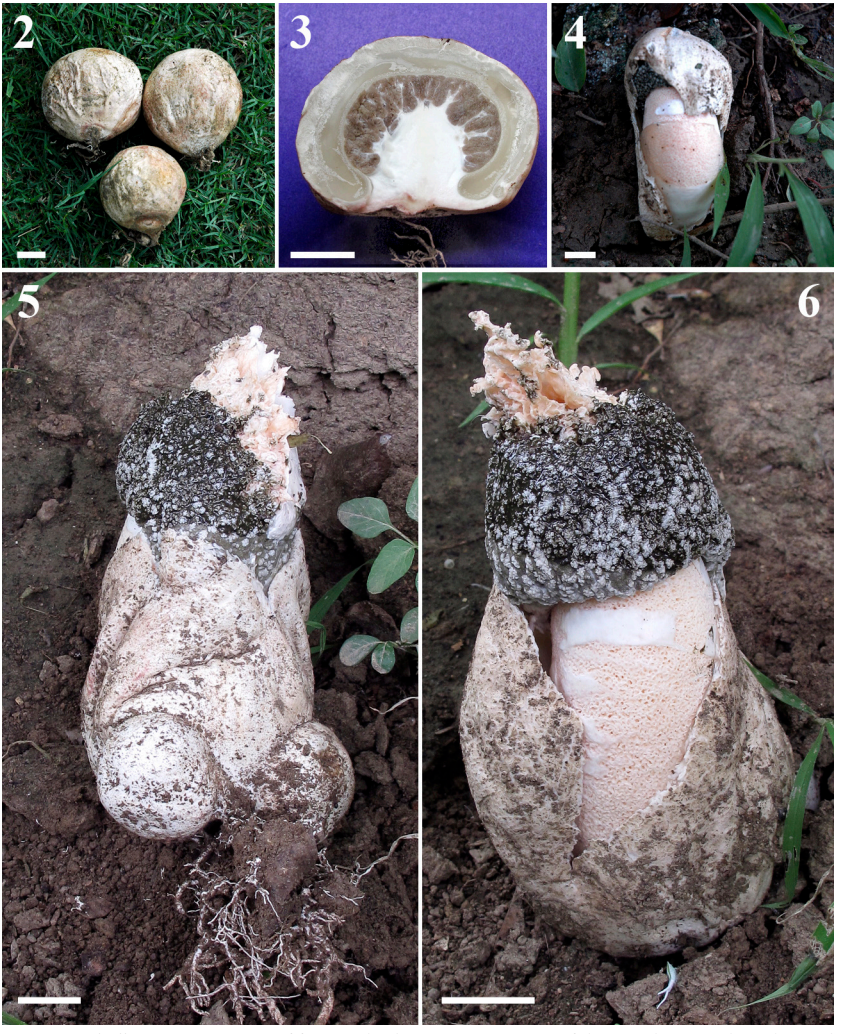
Egg ≤50 mm tall × 30 mm diam., subglobose to ovoid, sheathed by a tough, brown membrane. Mature fruiting body grayish to violet, ≤123 mm high, attached to the substratum by well-developed thick branched rhizomorphs at the base. Receptaculum with an off white reticulate pileus head and stipe. Pileus slightly campanulate, having glebal mass, ≤30 mm tall × 25 mm diam., cylindrical, narrowing at the top, hollow. Gleba olivaceous green wet, sticky, deliquescent, not very smelly. Stipe off white, spongy, hollow, porous, ≤80 mm tall × 17–20 mm diam., cylindrical, almost equal, firm, hard. Volva thick, texture soft, cup-shaped, covering 1/3rd of the stipe, globose, grayish to violet with off white tones, outer surface relatively smooth, not encrusted with soil particles, lined with grayish to violet gelatinous and sticky material inside, ≤37 mm tall × 35 mm diam. Spores 3–5 × 1-2 µm, ellipsoid, hyaline, smooth.

Material examined: **PAKISTAN**: Khyber Pakhtun Khwa, Khanspur, near P.U. teachers suites, ca. 2250 m a.s.l., solitary, on ground, on mountainous slopes, in Himalayan moist temperate forest, 22 Aug. 2010, N. Yousaf NYG01 (LAH 228210; AH 39161, GenBank KF481956).

Phallus roseus Delile, Descr. Egypte, Hist. Nat. 2: 300 (1813) Figs 2–6

≡ *Itajahya rosea* (Delile) E. Fisch., Ber. Deutsch. Bot. Ges. 47: 294 (1929)

Egg off white to pinkish, subglobose, outer surface rough, encrusted with soil particles, 55 mm tall × 75 mm diam.; longitudinal section showing three layers forming a semicircle: outer layer 6mm thick, grayish with off white tones; middle layer ≤9 mm wide, grayish, smooth, shiny, wet, sticky, inner layer brownish, roughened by finger-like projections facing the centre; core white, soft, covered by layers on all sides except at the base. Mature Fruiting Body



Figs 2-6: *Phallus roseus:* 2. Eggs of fruiting body. 3. Cross-section of the egg showing three-layered arrangement. 4–5. Fruiting body development. 6. Mature fruiting body. Bars = 20 mm.

gregarious, ≤80 mm tall × 17 mm diam at the top and 45 mm at the base, attached to the ground by well-developed, branched, white, thick rhizomorphs. Receptaculum bearing pileus and stipe. Pileus globose to subglobose, 21 mm high × 34 mm diam., hollow, having glebal mass with irregular appendices pinkish. Gleba grayish, turning blackish with age, unpleasant odor when collected. Soft, wet, sticky. Stipe off pinkish white to pinkish, hollow, spongy, porous, hard, entirely enclosed by volva, broader and wider at the base

(30 mm in diam.) and narrowing upwards (25 mm diam.), some stipe flesh present at the top of receptaculum as an extension, extended part irregularly shaped, pinkish, ≤17 mm high. Volva ovoid, saccate, 45 mm tall × 30–45 mm diam., prominent, well developed, covering all the stipe almost from all sides, ends where glebal mass is present, whitish, thick, texture soft, outer surface rough, slightly having patchy appearance, encrusted with soil particles, smooth, membranous, gelatinous and shiny from inside, remnants of volva present on the stipe in form of white patches. Spores 3–4 × 1.5-2 µm, ellipsoid, hyaline, smooth.

Material examined: **PAKISTAN**: Punjab, Lahore, University of the Punjab, Quaid-e-Azam campus, Plant nursery, RO II, ca. 217 m a.s.l., gregarious, on ground, under *Dalbergia sissoo* Roxb. (*Fabaceae*), 10 Aug. 2010, N. Yousaf NYG02 (LAH 108210, GenBank KF481955; AH 39160).

Discussion

Phallus hadriani, currently included in *Phallus* sect. *Dictyophora* (Desv.) Kreisel, is characterized by its reticulate pileus surface with pinkish or violet tones. It somewhat resembles *P. impudicus*, a species with a whitish volva that lacks the purple or pinkish purple tones typical of *P. hadriani*. Kreisel (1996)

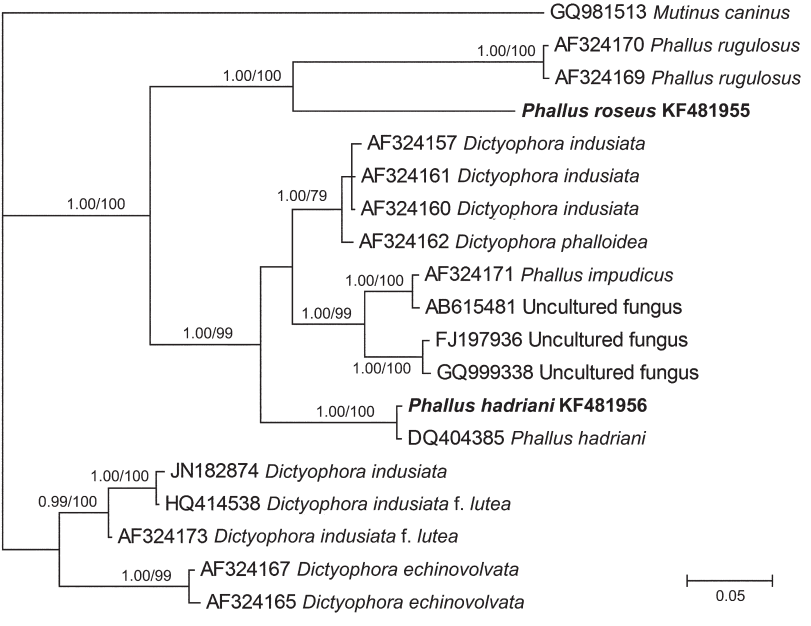


Fig. 7: Consensus ITS tree generated in MrBayes 3.1 showing the examined specimens and their closest relatives in the *Phallaceae*. Values above nodes represent PAUP maximum parsimony bootstrap proportions, while those below nodes represent MrBayes posterior probabilities.

cites *P. hadriani* from temperate and Mediterranean Europe, central Asia, China, Japan, and North America.

Although Fischer (1929) transferred *P. roseus* to *Itajahya,* Kreisel (1996) includes the species in *Phallus* subg. *Itajahya* (Möller) Kreisel, together with *P. galericulatus* (Möller) Kreisel and *P. glutinolens* (Möller) Kuntze (emend. Trierveiler-Pereira et al. 2009). *Phallus glutinolens* can be separated from both *P. roseus* and *P. galericulatus* based on its pinkish stipe and greenish gleba; *P. galericulatus* does not present a greenish gleba. *Phallus roseus,* a rare species from desert areas, was recently recorded from the Hadramout valley in Yemen (Kreisel & Al-Fatimi 2008) and the semi-arid Brazilian region (Ottoni et al. 2010).

We provide the first molecular data for *P. roseus*, which clearly separate the species from the *impudicus*/*hadriani* group and apparently link it to *P. rugulosus* Lloyd. Additionally, molecular data suggest that a cryptic species could exist within *P. impudicus*, as shown by the different lineages observed in the phylogenetic tree. However, the lack of sequences coming from available fruiting bodies make necessary further sampling efforts to confirm this hypothesis.

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